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Circadian phase and intertrial interval interfere with social recognition memory ☆

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ABSTRACT

A modified version of the social habituation/dis-habituation paradigm was employed to examine social recognition memory in Wistar rats during two opposing (active and inactive) circadian phases, using different intertrial intervals (30 and 60 min). Wheel-running activity was monitored continuously to identify circadian phase. To avoid possible masking effects of the light–dark cycle, the rats were synchronized to a skeleton photoperiod, which allowed testing during different circadian phases under identical lighting conditions. In each trial, an infantile intruder was introduced into an adult's home-cage for a 5-minute interaction session, and social behaviors were registered. Rats were exposed to 5 trials per day for 4 consecutive days: on days 1 and 2, each resident was exposed to the same intruder; on days 3 and 4, each resident was exposed to a different intruder in each trial. The resident's social investigatory behavior was more intense when different intruders were presented compared to repeated presentation of the same intruder, suggesting social recognition memory. This effect was stronger when the rats were tested during the inactive phase and when the intertrial interval was 60 min. These findings suggest that social recognition memory, as evaluated in this modified habituation/dis-habituation paradigm, is influenced by the circadian rhythm phase during which testing is performed, and by intertrial interval.

1. Introduction

Rhythmicity is a general feature of behavioral and physiological variables. Even complex processes such as learning and memory express different kinds of temporal modulation. Simple learning processes such as habituation to sound in pigeons [1] or to an open field in mice [2] are affected by the phase of the light–dark cycle during which testing is performed. More complex learning processes are also modulated by time. For instance, the performance of rats in passive and active avoidance tasks can vary according to the time of testing [3,4], and similarly, performance in the water maze task may differ between the active and inactive phases [5]. In mice, maze performance [6] and context fear conditioning [2,7] show a clear time-of-day effect. A common timing phenomenon in cognitive processes is time-stamping in which performance is better when testing is performed at the same time of day as training. This phenomenon has been observed in different learning tasks, including active avoidance [8], passive avoidance [8–10], appetitive learning [11], conditioned place preference [12,13] and place aversion [14].

It is thus of interest to establish the temporal dynamics of learning tasks commonly used in cognitive sciences. Social recognition memory in rats has been proposed to represent an interesting model for studies of autism spectrum disorders (*e. g.*, [15]). This type of memory has been investigated using an intruder–resident paradigm in which the social behavior of a resident animal directed towards an intruder is evaluated. The typical paradigm consists of two 5-minute exposure periods to either the same or to different intruders [16,17]; social memory is demonstrated when the resident animal spends more time investigating novel conspecifics than familiar intruders. In the original description of the test, Thor and Holloway [16] varied the interval between the two sessions. This and subsequent experiments revealed that the animals could recognize the intruder in the second session only when the inter-exposure interval was less than 30–40 min [18–24].

Another technique used to evaluate social learning is the habituation/dis-habituation task (*e. g.*, [25–27]) in which an animal is repeatedly exposed to the odor of a particular individual, which should result in habituation, before being exposed to the odor of a novel individual; this latter exposure may lead to dis-habituation. Burman and Mendl [28] used a modification of this task, considering the decrease in investigation of a juvenile by an adult as an index

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of habituation. Sekiguchi et al. [22] described the occurrence of habituation after six bouts of 5-minute exposures, separated by 10-minute intervals, also demonstrating that the habituation effect disappeared after a 24-hour interval.

An alternative to the above paradigms is the social discrimination test in which two intruders rather than one are presented to the resident in the second session: the familiar intruder presented in the first session, together with a new intruder [17]. Reijmers et al. [29] used this paradigm to analyze social recognition memory taking into account the temporal phase in which the animals were evaluated. Rats were tested at four different time points of the light–dark cycle (at *zeitgeber* times 3, 9, 15 and 21; respectively, ZT03, ZT09, ZT15 and ZT21) with an intertrial interval (ITI) of either 10 or 25 min. The authors found no temporal modulation of social recognition memory.

The present study further analyzes this issue by evaluating rats in two opposing phases of the circadian locomotor activity rhythm, at *zeitgeber* times 2 (ZT02) and 14 (ZT14) in a modified version of the habituation/dis-habituation paradigm. We also aim to extend previous work by focusing on the social investigatory behavior of the laboratory rat, particularly the habituation of investigative behavior after repeated presentations of the same and different conspecifics, testing the subjects at intertrial intervals of 30 or 60 min.

2. Materials and methods

2.1. Animals

Thirty-three, 8 week-old, male Wistar rats, weighing about 250 g, were purchased from the School of Medicine of the University of São Paulo. On arrival in the laboratory the animals were housed together (4–5 per cage) in the animal facility under a 12 h light:12 h dark (LD) cycle (lights on at 0600 h). Temperature was held at 21±2 °C. Food (Nuvilab) and water were provided *ad libitum*. The rats were divided into two groups: one tested during the active phase (ZT14), the other during the inactive phase (ZT02). Each group was subdivided according to testing session ITI (30 or 60 min). A further 120 young rats (about 25 days old), weighing from 50 to 100 g, housed in groups (8 per cage) held under the same conditions as the adults, were used as intruders to elicit social behaviors in the adult animals. Young animals were used rather than adults to avoid sexual or aggressive behaviors [16].

All procedures and animal care at the Laboratory for Neuroscience and Behavior of the Biosciences Institute of the University of São Paulo, complied with the Institute's guidelines, which conform to national and international standards and policies.

2.2. Light cycles and wheel-running activity recording

After 2 weeks of adjustment to laboratory conditions, the adult animals were placed in individual home-cages (46×25×38 cm) with computer-monitored running wheels (30 cm diameter, 10 cm width, 0.5 cm between bars). Cages were held in ventilated wooden cabinets (180×55×50 cm) and maintained on a 12 h:12 h LD cycle. Two weeks later, the 12 h dark phase was replaced by 12 h of dim light (Light:Dim cycle). The dim light (15–25 lx) was provided by three 100 W incandescent lamps connected to a dimmer (Exatron Exata 500 W); intense light (400–500 lx) was provided by two 30 W fluorescent lamps. When the activity rhythms were stable and precisely synchronized to the Light:Dim cycle, a skeleton photoperiod was established [30] consisting of two 30-minute bright light pulses separated by 11 h or 12 h of dim light (*i. e.*, 30 min bright light:11 h dim light, or 30 min bright light:12 h dim light). This condition was continued for the duration of the experiment. The reason for using this type of synchronizing agent was to allow testing at different circadian time points, under identical illumination conditions. After 7 days in the skeleton photoperiod, behavioral testing started.

Wheel-running activity was continuously recorded using an in-house computer program. Each turn of the wheel activated a micro-switch that was registered as one pulse of activity. The resulting data were analyzed and visualized using ClockLab software (Actimetrics, Inc., Evanston, IL). The activity data were plotted as actograms and were used to monitor entrainment to the Light:Dim and skeleton photoperiod cycles, and to confirm that testing took place during the desired circadian phases.

Behavioral testing was performed within the resident animals' home-cages. During each test, a camera (Sony CCD) was adapted on top of the cage (40 cm above the cage floor) where it remained throughout the entire session. The camera was connected to a 29″ TV apparatus (Philips) to monitor the session, and to a video system (Philips VR456/78) for behavioral recording.

2.3. Behavioral task

A modified version of the habituation/dis-habituation paradigm was used in association with Thor and Holloway's social recognition test [16,22,28]. Each trial began when a young intruder was introduced into the resident's home-cage for a 5-minute interaction session. At the end of the trial, the intruder was removed and returned to an individual holding cage. Each day, every resident was subjected to 5 trials; ITI was 30 or 60 min, depending on the group. On the first and second days the residents were exposed to the same intruder. On the third and fourth days a different intruder was presented to the residents in every individual trial.

This treatment allowed us to evaluate (1) whether long-term decrease in social investigation takes place from 1 day to the next (long-term habituation), both when the same intruder is presented (from the first to the second days) and when different intruders are presented (from the third to the fourth days); (2) whether short-term decrease in social investigation occurs from one trial to the next on any particular day (short-term habituation); (3) whether repeated exposure to either the 'same intruder' or to 'different intruders' results in habituation; and (4) if any of the above processes are affected by intertrial interval and/or by phase of the circadian rhythm.

The residents' behaviors measured were those oriented towards the intruder and included (1) sniffing the anogenital region, (2) sniffing the head, (3) sniffing the body, (4) following the intruder, (5) dominance (the resident handling the intruder whose back is on the floor) and (6) aggression (only behaviors that did not injure the intruder, *e. g.*, kicks) [18,19].

The trials were videotaped and the behaviors exhibited by the resident rats were subsequently scored. A computer-assisted data acquisition system allowed an experimenter unaware of the treatments to quantify the time spent by each resident in performing each behavior, which was then summed (dominance and aggression were not included) to provide the total duration of social investigatory behaviors. Resident rats that exhibited behaviors such as biting or any other behavior that might injure the intruder were excluded from the experiment. During the behavioral tasks, mainly the residents ran on the wheels.

2.4. Data analysis

The sum of the social *investigatory* behaviors was analyzed using a five-way ANOVA with three repeated measures. 'Circadian Phase' (ZT14 and ZT02) and 'ITI' (30 or 60 min) were the *between-subjects* factors. The within-subjects factors were 'Intruder' (Same Intruder on the first and second days, and Different Intruders on the third and fourth days), 'Day' (either first and second days with the same intruder, or first and second days with a different intruder; note that the latter correspond to the third and fourth days of testing), and 'Trial' (1 to 5 per day) (SAS Institute, Inc. Cary, NC). Additionally, separate ANOVAs were performed for each social behavior scored. *Post hoc* comparisons,

when required, were performed using Duncan's test; differences were considered significant at $p<0.05$.

3. Results

Fig. 1 provides representative actograms of a rat tested during its active phase (ZT14, top panel) and of another rat tested during its inactive phase (ZT02, lower panel). An activity rhythm synchronized to the external LD cycle (days 1 through 12) and to the subsequent Light:Dim cycle (days 13 through 25) is present. Wheel-running activity was concentrated mainly during the dark and dim phases. When the Light:Dim cycle was replaced by the skeleton photoperiod (day 26), entrainment continued. Once behavioral testing began with exposure of the resident animal to an intruder, a masking component appeared, *i. e.*, immediately after the intruder session, the resident rats ran intensively on their wheels (Fig. 1) (masking is considered to occur when an external agent–exposure to the social experience in the present case–produces a direct effect on wheel-running activity, without exerting its effect through the biological clock). This occurred in rats tested in both phases. In animals tested during their active phase, wheel-running activity was more intense compared to that seen during the corresponding phase of undisturbed days. In contrast, rats tested during their inactive phase exhibited an intense bout of post-test activity. Despite this masking effect, synchronization with the skeleton photoperiod appeared to continue and, consequently, we considered that testing was performed at the circadian time points planned.

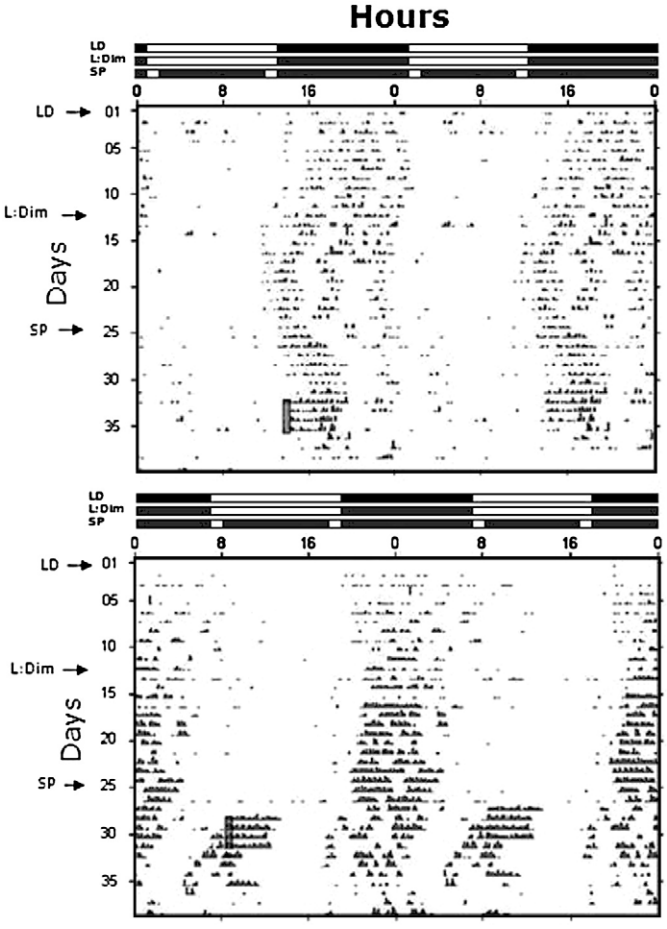

Fig. 1. Representative actograms of an animal tested during its active phase (top panel) and another tested during its inactive phase (lower panel). Time is plotted across the horizontal axis (48 h per line), and successive days are plotted beneath one another. The uppermost bar indicates the light–dark (LD) cycle to which the animals where initially submitted during days 1 through 12. The middle bar indicates the light–dim cycle from days 13 to 25, while the lower bar indicates the skeleton photoperiod to which the animals were subsequently submitted. The horizontal arrows to the left indicate when each cycle was begun. The vertical bars within the actograms indicate when behavioral testing was performed.

Fig. 2 shows time spent in social investigatory behaviors by the resident rats when tested during the inactive (ZT02; left graph) and active (ZT14; right graph) phases of their circadian rhythm, over four testing days using five trials per day and 30 or 60 min ITIs. On the first and second days, the resident rats were exposed to the same intruder (left side of each graph) and on the third and fourth days they were exposed to different intruders in each trial (right side of each graph). Note that for statistical purposes, the third and fourth days were treated like the first and second days with different intruders.

Long-term habituation was not seen, either to the same intruder (first and second days) or to different intruders (third and fourth days). That is, during the early trials of the second testing day with the same intruder, the resident rats exhibited greater social investigation compared to the late trials of the previous day; this effect was independent of testing phase. When comparing the third and fourth days with different intruders in each single trial, no general decrease in social investigation was seen in either phase. The ANOVA revealed a significant Intruder × Day × Trial × Phase interaction effect ($F(4,116)=8.37$; $p<0.0001$).

Short-term habituation of social investigation was mainly seen when the resident rats were exposed to the same intruders; this effect was affected by phase. The ANOVA revealed a significant Day ($F(1,29)=4.75$; $p<0.04$), and significant Intruder × Day ($F(1,29)=16.53$; $p<0.0003$) and Intruder × Trial ($F(4,116)=5.91$; $p<0.0002$) interaction effects (see additional ANOVA results below). Fig. 2 shows that when exposed to the same intruder over repeated trials within a single day, the resident rats exhibited a decrease in social investigatory behaviors expressing habituation to the same intruder. Conversely, when exposed to different intruders, the resident rats showed no such decrease in social investigation. Note that this habituation effect to repeated exposure to the same intruder during the first testing day was stronger for animals tested during their inactive phase (compare left and right panels for day 1).

These results also reveal a clear intertrial interval effect that was affected by phase of circadian rhythm. That is, resident rats tested in their inactive phase exhibit greater social investigation towards different intruders when ITI was 60 min, compared to an ITI of 30 min (the ANOVA revealed a significant Trial × Phase × ITI interaction effect—$F(4,116)=2.66$; $p<0.004$, and an almost significant Intruder × Phase × Interval interaction effect—($F(1,29)=3.92$; $p=0.0573$) (Fig. 2, left panel). This effect did not occur in resident rats tested during their active phase; *i. e.*, social investigation by these rats when tested using either 30 or 60 min ITIs did not differ (Fig. 2, right panel). During the first trial of day 1 there were (1) significant differences between subjects tested during their inactive phase as compared to subjects of corresponding groups tested in their active phase (Duncan's test, 30-minute ITI, $p=0.0164$, and 60-minute ITI, $p=0.0169$) and (2) no significant differences between subjects exposed to different ITIs tested either in their inactive (Duncan's test, $p=0.184$) or in their active (Duncan's test, $p=0.933$) phases.

Exposure to different intruders induced significantly greater social investigation compared to exposure to the same intruder (Intruder effect, $F(1,29)=117.40$; $p<0.0001$). This effect was seen both in rats tested during their inactive phase (Fig. 2, left panel) and those tested during their active phase (Fig. 2, right panel).

In addition to analyzing the sum of all social investigatory behaviors, each previously defined social behavior was analyzed separately (data not shown). Many of these behaviors showed significant phase effects including anogenital investigation (Trial × Phase interaction effect: $F(4,116)=4.21$; $p<0.01$; Intruder × Day × Trial × Phase interaction effect: $F(4,116)=2.34$; $p<0.05$; Trial × Phase × ITI interaction effect:

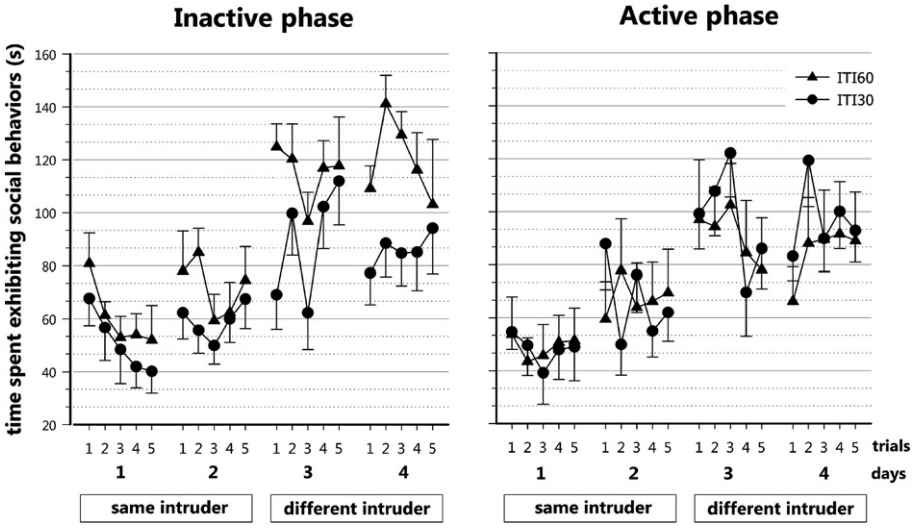

Fig. 2. Mean (±SEM) duration in seconds of social behaviors exhibited by resident rats in each of five trials per day, during four consecutive days. During the first and second days, the resident rats were exposed to the same intruder; during the third and fourth days they were exposed to a different intruder in each trial. Two groups of animals were tested during their inactive phase (left panel) and another two groups during their active phase. In each case, one group of animals was tested with a 60-minute ITI (▲) and one group with a 30-minute ITI (●).

$F(4,116)=3.51$; $p<0.01$; Intruder×Day×Trial×Phase interaction effect: $F(4,116)=6,35$; $p<0.0001$), sniffing the intruder's body (Intruder×Trial×Phase×ITI interaction effect: $F(4,116)=2.90$; $p<0.05$; Intruder×Day×Trial×Phase interaction effect: $F(4,116)=2.62$; $p<0.05$), following the intruder (Intruder×Phase interaction effect: $F(1,129)=4.99$; $p<0.05$; Intruder×Trial×Phase×ITI interaction effect: $F(4,116)=3.44$; $p<0.01$) and dominance behavior (Intruder×Phase interaction effect: $F(1,29)=4.26$; $p<0.04$; Intruder×Phase×ITI interaction effect: $F(1,29)=4.59$; $p<0.05$; Intruder×Day×Trial×Phase×ITI interaction effect: $F(4,116)=2.34$; $p<0.05$). In general, the time spent by the resident rats exhibiting these behaviors was greater when tested during their inactive phase and when the ITI was 60 min. More than 50% of the time spent exhibiting social investigatory behaviors corresponded to anogenital investigation. Thus, the effects represented in Fig. 2 correspond largely to anogenital investigation. When this behavior is analyzed separately, the data are very similar to those for total social investigatory behavior.

4. Discussion

This study employed a modified version of the habituation/dishabituation paradigm to investigate the effects of circadian phase and intertrial interval on social recognition memory. The parameters evaluated were social investigation towards conspecifics following repeated exposures to the same intruder as compared to repeated exposures to different intruders. The main findings suggest that resident's social investigatory behavior was more intense when different intruders were presented compared to repeated presentation of the same intruder, suggesting social recognition memory. This effect was stronger when the rats were tested during the inactive phase and when the intertrial interval was 60 min.

As expected, the resident rats did not exhibit long-term habituation, either when exposed to the same intruder or when exposed to different intruders, independently of testing phase. That is, habituation to an intruder after five, 5-minute exposure sessions on the first day is not transferred to the second day of testing with the same intruder. This occurred even considering the fairly long exposure to the same intruder (25 min divided into 5 trials of 5 min each). This finding replicates earlier data showing that rat's social recognition resembles short-term memory [18–24].

Short-term habituation of social investigation was seen after repeated exposure to the same intruder; this process was affected by phase. That is, when exposed to the same intruder over repeated trials within a single day, the resident rats exhibited a decrease in social investigatory behaviors expressing habituation to the same intruder; this finding suggests social memory. Conversely, when exposed to different intruders, the resident rats showed no such decrease in social investigation. Together these results suggest that the resident rats recognized a previously presented juvenile, maintaining this information in their memory during the intertrial interval.

The phase of circadian rhythm influenced the intertrial interval effects on social investigation. That is, resident rats tested in their inactive phase showed greater social investigation towards intruders when ITI was 60 min compared to an ITI of 30 min; this effect was not seen in resident rats tested during their active phase (Fig. 2). On the other hand, rats exhibited lower levels of social investigation towards the same intruder on the second trial of the first day of testing (see Fig. 2) independently on phase and of ITI. Thus, social recognition memory function does not appear to have failed. These data suggest that the greater social investigation towards different intruders seen in resident rats tested during their inactive phase when ITI was 60 min reflects the cumulative effect of repeated exposures initially to the same intruder (on the first and second days of testing) and then to different intruders (on the third and fourth days of testing) (see below).

It is likely that social interactions occur during the rats' active phase [31]. Thus, the appearance of an intruder during the residents' inactive phase, a period during which encounters are unexpected, may intensify the reaction to this social stimulus and hence lead to greater social investigation, at least in the earlier trials of exposure to the intruder. While this interpretation explains the increased social investigation when resident rats are tested in their inactive phase, it does not explain why this effect is restricted to rats tested with a 60-minute ITI. However, it is well known that repeated exposure to the same stimulus leads to habituation, an effect that increases as ITI decreases [32–34].. Thus, these animals show less habituation to the social stimulus when ITI is longer (see Fig. 2, left panel, day 1), an effect carried over to testing with different intruders (Fig. 2, left panel) (see below). Note, however, that this latter interpretation does not apply to the results for rats tested in their active phase (Fig. 2, right panel), since the amount of social investigation seen here is independent of ITI. Thus, there may be an association between both intensification of the reaction to social stimulus when the test is

performed in the inactive phase and slower habituation associated with a longer ITI.

Exposure to different intruders induced significantly greater social investigation compared to exposure to the same intruder (Fig. 2), suggesting that the resident rats recognized previously encountered intruders, independently on phase of testing. These results confirm previous literature reports [29].

Repeated exposures to the same intruder lead to habituation (Fig. 2), as predicted by Staddon et al. [33] model of habituation, memory decay, and interval timing. On the second day, the level of social investigation increased indicating that the interposition of a 24-hour interval leads to "dis-habituation" and, further, to intensified social investigation. This latter effect may be related to non-photic synchronization, *i. e*., a time-related increase in the animals' general activity in anticipation of a relevant event [35] which, in the present case, corresponds to the social encounters. In fact, the actograms of animals tested during their inactive phase reveal an increase in activity level just before, and just after testing; this effect was already visible on the day following the first social encounter, becoming stronger as testing proceeded over time (see a representative example in Fig. 1, lower panel). Similar circadian effects have been described in rats trained in the Morris water maze task [36], indicating that social encounter is also a significant arousing experience. Synchronization to social stimuli is well known (see [35]). Such intensification of activity level may have contributed to the increase in social investigation particularly when the animals were tested during their inactive phase (see [1,37]).

The production and release of pheromones may be circadian (see [38]). Since social behavior in rodents is mainly based on olfactory cues [19,24], a circadian rhythm of pheromone production may explain the effect of time on social behavior in these animals. This issue remains to be evaluated.

The present findings do not conflict with those of Reijmers et al. [29] who reported that social recognition memory is not influenced by circadian phase when the second trial is performed either 10 or 25 min after the first session. Our study shows that there were no phase effects on social investigation when ITI was 30 min; however, our data did reveal phase effects when ITI is increased to 60 min. Reijmers' experiments used many methodological differences compared to the present study; *e. g*., their rats were entrained to a standard light–dark cycle, and thus, groups tested in different phases were tested under different lighting conditions. Light exerts an inhibitory effect on the expression of behaviors by nocturnal animals [39]. Our use of a skeleton photoperiod allowed testing during different circadian phases but under exactly the same lighting conditions, thus avoiding both the aversive effects of light and the facilitatory effects of darkness. This protocol also unmasked the influence of circadian phase on social investigatory behavior.

The 30- and 60-minute ITI protocols used here entail considerable differences in duration of the daily experimental procedures. For a 30-minute ITI, the experiment lasted 2 h 25 min per day; for 60 min, the daily experiments required 4 h 25 min. This difference implies a longer interference interval, the consequences of which may be dissimilar when performed during a phase in which sleeping is preferred. The actograms of animals tested using a 60-minute ITI during their inactive phase display an activity pattern less organized than those of rats tested using a 30-minute ITI. To what extent this difference may have affected performance is not clear.

Our findings appear to conflict with the literature suggesting that social memory of a single 5-minute social encounter lasts less than 40 min [20–24]. That is, substantial decrease in social investigation towards the same intruder was seen for residents tested in their inactive phase when the time interval between encounters was 60 min. One explanation for this finding is that testing during the inactive phase improves social memory. Congruent with this interpretation, most studies on social memory have typically tested animals during their active phase [18–24]; the present results also show shorter social memory when rats were tested using a 60-minute ITI during their active phase. This repeated social stimulation during the inactive phase with a 60-minute ITI may facilitate maintenance of social information.

The resident animals in the present study were housed individually in cages provided with running wheels for 1 month before social behavioral testing began. Exercise is known to improve performance in certain learning tasks (*e. g*., [40]). Such animals may be able to consolidate their memories better, and evaluation of this hypothesis requires further investigation. However, these rats were also isolated for at least 1 month before beginning testing. Social isolation in rats can lead to several behavioral changes such as increased aggression and avoidance, as well as anxiety behaviors and working memory impairments among other factors like neuroendocrine alterations [41–43]. Our animals may be exhibiting alterations in social investigatory behavior, improving their ability to recognize a familiar conspecific, even when the intertrial interval is as long as 60 min, *i. e*., even though social isolation may negatively affect memory in some tasks, those that evaluate sociability may be affected differently by social isolation. Since our animals had been isolated for a lengthy period, their altered reaction to conspecifics may have led to a differential capability to remember such interactions. Similarly, alterations due to social isolation may underlie the finding that animals tested during their inactive phase and submitted to a 60-minute intertrial interval exhibit more intense social investigation.

In summary, the performance data generated by this adapted habituation/dis-habituation paradigm under the experimental conditions used here, suggest that the temporal modulation of social investigatory behaviors takes place mainly when the animals are tested using a 60-minute intertrial interval. These temporal differences in social performance should be carefully considered when analyzing data from animals tested at different points of the circadian cycle.

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References

[1] Valentinuzzi VS, Ferrari EA. Habituation to sound during morning and night sessions in pigeons (*Columba livia*). Physiol Behav 1997;62:1203–9.
[2] Valentinuzzi VS, Kolker DE, Hotz Vitaterna M, Ferrari EAM, Takahashi JS, Turek FW. Effect of circadian phase on context and cued fear conditioning in C57BL/6J mice. Anim Learn Behav 2001;29:133–42.
[3] Davies JA, Navaratra V, Redfern PH. A 24-hour rhythm in passive-avoidance behavior in rats. Psychophysiology (Berl) 1973;32:211–4.
[4] Catalá MD, Pallardo F, Roman A, Villanueva P, Viña Giner JM. Effect of pinealectomy and circadian rhythm on avoidance behavior in the male rat. Physiol Behav 1985;34:327–33.
[5] Valentinuzzi VS, Menna-Barreto M, Xavier GF. Effect of circadian phase on performance of rats in the Morris water maze task. J Biol Rhythms 2004;19:312–24.
[6] Hoffmann HJ, Balschun D. Circadian differences in maze performance of C57BL/6 mice. Behav Process 1992;27:77–84.
[7] Chaudhury D, Colwell CS. Circadian modulation of learning and memory in fear-conditioned mice. Behav Brain Res 2002;133:95–108.
[8] Holloway FA, Wansley R. Multiple retention deficits at periodic intervals after active and passive avoidance. Behav Biol 1973;9:1–14.
[9] Holloway FA, Wansley R. Multiphasic retention deficits at periodic intervals after passive-avoidance learning. Science 1973;180:208–10.
[10] Wansley RA, Holloway FA. Oscillations in retention performance after passive-avoidance training. Learn Motiv 1976;7:296–302.
[11] Wansley RA, Holloway FA. Multiple retention deficits following one-trial appetitive training. Behav Biol 1975;14:135–49.
[12] Cain SW, Ko CH, Chalmers JA, Ralph MR. Time of day modulation of conditioned place preference in rats depends on the strain of rat used. Neurobiol Learn Mem 2004;81:217–20.
[13] Valentinuzzi VS, Carneiro BT, Santana KS, Araujo JF, Ralph MR. Memory for time of training modulates performance on a place conditioning task in marmosets. Neurobiol Learn Mem 2008;89:604–7.
[14] Cain SW, Chou Tina, Ralph MR. Circadian modulation of performance on an aversion-based place learning task in hamsters. Behav Brain Res 2004;150:201–5.

[15] Marin JC, Moura PJ, Cysneiros RM, Colugnati DB, Cavalheiro EA, Scorza FA, et al. Temporal lobe epilepsy and social behavior: An animal model for autism? Epilepsy Behav 2008;13:43–6.
[16] Thor DH, Holloway WR. Social memory of the male laboratory rat. J Comp Physiol Psychol 1982;96:1000–6.
[17] Engelmann M, Wotjak CT, Landgraf R. Social discrimination procedure: an alternative method to investigate juvenile recognition abilities in rats. Physiol Behav 1995;58:315–21.
[18] Dantzer R, Koob GF, Bluthe RM, Le Moal M. Septal vasopressin modulates social memory in male rats. Brain Res 1988;457:143–7.
[19] Dantzer R, Bluthe RM, Koob GF, Le Moal M. Modulation of social memory in male rats by neurohypophyseal peptides. Psychopharmacology (Berl) 1987;91:363–8.
[20] Popik P, Wolterink G, De Brabander H, van Ree JM. Neuropeptides related to [Arg8] vasopressin facilitates social recognition in rats. Physiol Behav 1991;49:1031–5.
[21] Sekiguchi R, Wolterink G, van Ree JM. Analysis of the influence of vasopressin neuropeptides on social recognition of rats. Eur Neuropsychopharmacol 1991;1: 123–6.
[22] Sekiguchi R, Wolterink G, van Ree JM. Short duration of retroactive facilitation of social recognition in rats. Physiol Behav 1991;50:1253–6.
[23] Engelmann M, Landgraf R. Microdialysis administration of vasopressin into the septum improves social recognition in Brattleboro rats. Physiol Behav 1994;55: 145–9.
[24] Popik P, van Ree JM. Neurohypophyseal peptides and social recognition in rats. Prog Brain Res 1998;119:415–36.
[25] Halpin ZT. Individual odors among mammals: origins and functions. Adv Study Behav 1986;16:40–70.
[26] Jonhston RE. Memory for individual scent in hamsters (*Mesocricetus auratus*) as assessed by habituation methods. J Comp Psychol 1993;107:201–7.
[27] Johnston RE, Jernigan P. Golden hamsters recognize individuals, not just individual scents. Anim Behav 1994;47:129–36.
[28] Burman O, Mendl M. The effects of environmental context on laboratory rat social recognition. Anim Behav 1999;58:629–34.
[29] Reijmers LG, Leus IE, Burdach JP, Spruijt BM, Van Ree JM. Social memory in the rat: circadian variation and effect of circadian rhythm disruption. Physiol Behav 2001;72:305–9.
[30] Pittendrigh CS. On the mechanism of entrainment of circadian rhythms by light cycles. Circadian clocks. Amsterdam: North Holland; 1965. p. 277–97.
[31] Sams-Dodd F. Effects of continuous D-amphetamine and phencyclidine administration on social behavior, stereotyped behavior, and locomotor activity in rats. Neuropsychopharmacology 1998;19:18–25.
[32] Thompson RF, Spencer WA. Habituation: a model phenomena for the study of neuronal substrates of behavior. Psychol Rev 1966;73:16–43.
[33] Staddon JER, Chelaru IM, Higa JJ. Habituation, memory and the brain: the dynamics of interval timing. Behav Processes 2002;57:71–88.
[34] Buhusi CV, Meck WH. What makes us tick? Functional and neural mechanisms of interval timing. Nat Rev Neurosci 2005;6:755–65.
[35] Mrosovsky N. Locomotor activity and non-photic influences on circadian clocks. Biol Rev 1996;71:342–72.
[36] Valentinuzzi VS, Diniz GP, Menna-Barreto L, Xavier GF. The experience in the water maze task can affect the circadian rhythm of locomotor activity. Biol Rhythm Res 2007;38:399–414.
[37] Valentinuzzi VS, Buxton OM, Chang AM, Scarbrough K, Ferrari EA, Takahashi JS, et al. Locomotor response to an open field during C57BL/6J active and inactive phases: differences dependent on conditions of illumination. Physiol Behav 2000;69:269–75.
[38] Jechura TJ, Stimpson CD, Lee TM. Odor-facilitated reentrainment in male and female juvenile *Octodon degus*. Physiol Behav 2006;89:617–22.
[39] Marques MD, Waterhouse JM. Masking and the evolution of circadian rhythmicity. Chronobiol Int 1994;11:146–55.
[40] van Praag H, Christie BR, Senowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A 1999;96: 13427–31.
[41] Wongwitdecha N, Marsden CA. Effects of social isolation rearing on learning in the Morris water maze. Brain Res 1996;715:119–24.
[42] Weiss IC, Pryce CR, Jongen-Relo AL, Nanz-bahr NI, Feldon J. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res 2004;52:279–95.
[43] Sandstrom NJ. Sex differences in the long-term effect of preweanling isolation stress on memory retention. Horm Behav 2005;47:556–62.